

Figure 1

Nucleotide sequence of the *Prunus amygdalus* HNL5 gene obtained by PCR amplification

The start codon (ATG) and stop codon of the open reading frame are printed in bold type, and the nucleotides in the intron regions are indicated in lower case letters. The peripheral sequences which have been attached via the PCR primers and which are not part of the HNL5 gene are underlined. The splice sites of the introns were identified with the aid of the consensus sequence "GT....AG".

1 GGAATTCACA ATATGGAGAA ATCAACAATG TCAGTTATAC TATTTGTGTT
51 GCATCTTCTT GTTCTTCATC TTCAGTATTC AGAGGTTTAC TCGCTTGCCA
101 ATACTTCTGC TCATGgtaaa ttccatctt cagtattcat ttaacagcaa
151 aatgtgtaga ttataatta agaaactga cacaagtagt gcaagaaaca
201 agctaattta gatgcatgtt gaaaaaaatc tticatctt tcacatatat
251 ttgcagATT TTAGCTACTT GAAGTTTGTG TACAACGCCA CTGATACAAG
301 CTCGGAAGGA TCATATGACT ACATTGTAAT CGGTGGAGGA ACATCAGGGT
351 GTCCATTGGC AGCAACTTTA TCAGAAAAAT ACAAGGTGCT TCTTCTAGAA
401 AGAGGCACTA TTGCTACAGA ATACCCGAAC ACGTTGACTG CAGATGGGTT
451 TGCATATAAT CTGCAGCAAC AAGATGATGG AAAGACGCCA GTTGAAAGGT
501 TCGTGTCCGA AGATGGCATT GATAATGTGC GAGCCAGGAT CCTCGGTGGC
551 ACGACCATAA TCAATGCAGG CGTCTACGCC AGAGCTAACA TTTCATTCTA
601 TAGTCAAACA GGAATTGAAT GGGACCTGGA TTTGGTCAAT AAGACATATG

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651 AGTGGGTTGA AGACGCCATT GTGGTCAAGC CAAATAATCA ATCTTGGCAA
701 TCTGTTATAG GAGAGGGATT CTTGGAGGCG GGTATTCTTC CAGACAATGG
751 ATTTAGTTTG GATCACGAAG CAGGAACTAG ACTCACCGGC TCAACTTTTG
801 ACAATAATGG AACGCGACAT GCGGCTGATG AACTGCTTAA TAAAGGAGAC
851 CCTAATAACT TGCTAGTTGC AGTTCAGGCC TCAGTAGAGA AGATCCTCTT
901 CTCTTCCAAT ACATCAAgta tgttgcata gtgatatta atggtagctc
951 ctagtgtgc atgctgcact cgaaaattat taitttatca ttttaaata
1001 ctaacagaat agtgtgaagt ccatattc cctccatat ttcccaaatt
1051 tccataaaca aaacttcca attcctctc gtttagttg acaataatta
1101 taagctattc tcaatgcag ATTTGTCAGC TATTGGAGTC ATATATACGG
1151 ATTCTGATGG AAACCTCAT CAGGCATTTG TACGCGGTAA CGGAGAAGTT
1201 ATTGTTAGTG CAGGGACAAT CGGAACGCCT CAGCTTCTAC TACTTAGTGG
1251 CGTTGGACCA GAGTCTTACC TATCTTCTCT CAACATCACA GTTGTTACGC
1301 CGAATCCTTA TGTTGGGCAG TTTGTGTATG ACAATCCTCG TAATTTTATT
1351 AATATTTTGC CCCCAAATCC AATTGAAGCC TCTGTTGTAA CTGTTTATAGG
1401 CATTAGAAGT GATTATTATC AAGTTTCTCT GTCAAGCTTG CCATTTTCCA
1451 CTCCACCCTT TAGTCTTTT CCTACAACAT CTTACCCCTT CCCAAATTCG
1501 ACTTTTGCTC ATATTGTTAG CCAAGTTCCA GGACCATTGT CTCATGGTTC
1551 TGTCACGCTA AATTCATCAT CTGACGTGAG AATCGCTCCA AATATTAAAT
1601 TCAATTACTA TTCAAATTCC ACAGACCTTG CTAATTGTGT TAGCGGCATG
1651 AAGAAGCTTG GTGACTTATT AAGGACAAAG GCATTAGAAC CATATAAAGC

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1701 TCGAGATGTG CTGGGAATTG ACGGTTTCAA TTATTTGGGA GTACCTTTGC
1751 CAGAGAACCA AACAGATGAT GCATCCTTCG AAACATTTTG TCTAGATAAT
1801 GTAGCTTCAT ACTGGCATT A CCACGGTGGA AGCCTTGTTG GGAAAGTGCT
1851 TGATGACAGT TTCCGTGTTA TGGGGATCAA AGCATTACGC GTTGTGATG
1901 CCTCCACTTT CCCTTACGAA CCAAACAGCC ATCCTCAGGG CTTCTATCTG
1951 ATGTTAGGAA Ggtatgtgat gcacactcc aaccactaga gattctcaat
2001 attttgttgt tgttgtaatg aactctctgc cgcattgctc tttttatta
2051 atccttaaaa ttttgttt tgccgagGTA TGTGGGCCTT CAAATCCTGC
2101 AAGAAAGGTC AATCCGGTTG GAGGCTATTC ATAATATTCA AGAGTCCATG
2151 TGAAGAAATTC CG

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Figure 2

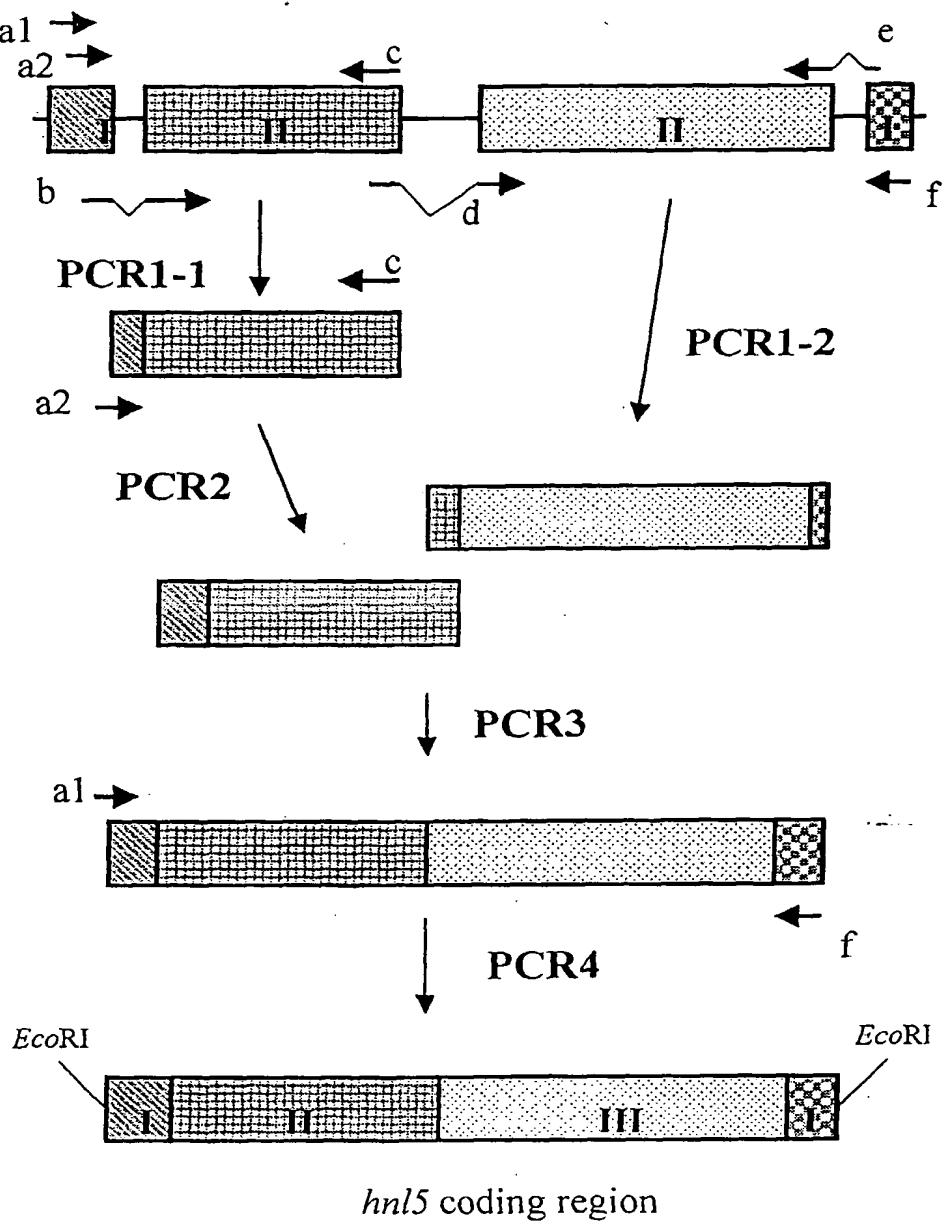


Figure 3:

Amino acid sequence of the *Prunus amygdalus* hydroxynitrile lyase (HNL5), derived from the nucleotide sequence of the HNL5 gene. The signal sequence determined from sequence analysis is printed in bold type and the postulated processing site is indicated by an arrow. Possible glycosylation sites (PROSITE patterns) are underlined.



MEKSTMSVILFVLHLLVLHLQYSEVHSLANTSAHDFS~~YLK~~FVYNATDTSSEGSYDYI
VIGGGTSGCPLAATLSEKYKVLLLERGTIATEYPNTLTADGFAYNLQQQDDGKTPVE
RFVSEGDIDNVRARILGGTTIINAGVYARANISFYSGTGIEWDL~~DLVN~~KTYEWVEDAI
VVKPN~~NQSW~~QSVIGEGFLEAGILPDNGFSLDHEAGTRLTGSTFDNNGTRHAADELL
NKGDPNNLLVAVQASVEKILFSSNTSNLSAIGVIYTDSDGNSHQAFVRGNGEVIVSA
GTIGTPQLLLLSGVGPESYLSSLNITV~~VQPN~~PYVGQFVYDNPRNFINILPPNPIEASVV
TVLGIRSDYYQVSLSSLPFSTPPFSLFPTTSYPLPNSTFAHIVSQVPGPLSHGSVTLN
SSSDVRIAPNIKFNYYSNSTDLANCVSGMKKLGDLLRTKALEPYKARDVLGIDGFNY
LGVPLPENQTD~~DDAS~~FETFCLDNVASYWHYHGGSLVGKVLDDSFVRVMGIKALRVVD
ASTFPYEPNSHPQGFYLM~~LG~~RYVGLQILQERSIRLEAIHNIQESM

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Figure 4:

Nucleic acid sequence of the DNA fragment coding for a secretory hybrid protein (PamHNL5xGOX) with HNL activity, consisting of sequences of the *Prunus amygdalus* HNL5 gene and the *Aspergillus niger* glucose oxidase gene.

gaattcatcatgcagactctccttgtagctcgcttggtctccctcgctgcggccctgcc-
cactacatcaggagcaatggcattgaagcctacaacgccactgatacaagctcggaaggatca-
tatgactacattgtaatcgggtggaggaacatcaggggtgccattggcagcaactttatcagaa-
aaatacaaggtgcttctctagaaagaggcactattgctacagaatacccgaaacacgtt-
gactgcagatgggittgcatataatctgcagcaacaagatgatggaaagacgccagttga-
aagggtcggtgccgaagatggcattgataatgtgcgagccaggatcctcggtggcacgacca-
taatcaatgcaggcgtctacgccagagctaacatttcattctatagtcaaacaggaatt-
gaatgggacctggatttggtcaataagacatatgagtgggtgaagacgccattgtggt-
caagccaaataatcaatcttggaatctgttataggagagggattcttgaggcggg-
tattctccagacaatggatttagttggatcacgaagcaggaactagactcaccggct-
caacttttgacaataatggaacgcgacatgcggctgatgaactgcttaataaaggagaccc-
taataacttgctagttgcagttcaggcctcagtagagaagatcctctctctccaatacat-
caaatttgctagctattggagtcetataacggattctgatggaaactctcatcaggcattg-
tacgcggtaacggagaagttatgttagtgacgggacaatcggaacgcctcagcttctac-
tacttagtggcgttgaccagagicttacctatctctcaacatcacagttgttcagcc-
gaatccttatgttgggcagtttgtatgacaatcctcgttaatttcattaatatttgcccc-
caaatccaattgaagcctctgttgaactgttttaggcattagaagtgattattat-
caagtttctgtgaagcttgccattttccactccacccttagtcttttctacaacatct-
taccctcccaaatcgacttttgctcatattgttagccaagttccaggaccattgtct-
catgggtctgtcacgctaaattcatcatctgacgtgagaatcgctccaaatattaaattcaat-
tactattcaaattccacagaccttgctaattgtgttagcggcatgaagaagcttggtgact-
tattaaggacaaaggcattagaacctataaagctcgagatgtgctgggaattgacggttt-
caattatttgggagtagctttgccagagaaccaaacagatgatgcaccttcgaaa-
cattttgtctagataatgtagcttcatactggcattaccacgggtggaagccttgttggga-
aagtgcttgatgacagtttccgtgttatgggatcaaagcattacgcgttgttgatgcctc-
cactttcccttacgaaccaaacagccatcctcagggcttctatctgatgttaggaagg-
tatgtgggccttcaaatcctgcaagaaagggtcaatgcagtgcgagcgccgcatgcgaattc

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Figure 5: Amino acid sequence of the hybrid protein PamHNL5xGOX, derived from the nucleic acid sequence (figure 4).

MQTLLVSSLVSLAAALPHYIRSNIEAYNATDTSSEGSYDYIVIGGGTSGCPLAATL
SEKYKVLLLERGTIATEYPNTLTADGFAYNLQQQDDGKTPVERFVSEDGIDNVRARI
LGGTTIINAGVYARANISFYSTGIEWDLVLNKTIEWVEDAIVVKPNNQSWQSVIG
EGFLEAGILPDNGFSLDHEAGTRLTGSTFDNNGTRHAADELLNKGDPPNLLVAVQA
SVEKILFSSNTSNLSAIGVIYTDSDGNSHQAFVRGNGEVIVSAGTIGTPQLLLLSGVG
PESYLSSLNITVWQPNPYVGQFVYDNPRNFILPPNPIEASVVTVLGIRSDYYQVSLS
SLPFSTPPFSLFPTTSYPLPNSTFAHIVSQVPGPLSHGSVTLNSSSDVRIAPNIKFNY
YSNSTDLANCVSGMKKLGDLLRTKALEPYKARDVLGIDGFNYLGVPLPENQTDDAS
FETFCLDNVASYWHYHGGSLVGKVLDDSFVRVMGIKALRVVDASTFPYEPNSHPQG
FYLMLGRYVGLQILQERSMO

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Figure 6: Comparison of the amino acid sequences of *Prunus amygdalus* HNL5 and of the hybrid protein PamHNL5xGOX. Sequence parts of *Aspergillus niger* glucose oxidase are underlined. Sequence regions having no significant homology between the two proteins are printed in italics, and the signal peptides are printed in bold type.

PamHNL5Gox 1 *mqtlivsslv vslaaalphy irsnqiea*-----YNATDTSS
PamHNL5 1 *mekstmsvil fvhlvlvhl qysevhsian tsahdfsylk fv*YNATDTSS

PamHNL5Gox 37 EGSYDYIVIG GGTSGCPLAA TLSEKYKVLL LERGATIEY PNTLTADGFA
PamHNL5 51 EGSYDYIVIG GGTSGCPLAA TLSEKYKVLL LERGATIEY PNTLTADGFA

PamHNL5Gox 87 YNLQQQDDGK TPVERFVSED GIDNVRARIL GGTTIINAGV YARANISFYS
PamHNL5 101 YNLQQQDDGK TPVERFVSED GIDNVRARIL GGTTIINAGV YARANISFYS

PamHNL5Gox 137 QTGIEWDLDL VNKTYEWVED AIVVKPNNQS WQSVIGEGFL EAGILPDNGF
PamHNL5 151 QTGIEWDLDL VNKTYEWVED AIVVKPNNQS WQSVIGEGFL EAGILPDNGF

PamHNL5Gox 187 SLDHEAGTRL TGSTFDNNGT RHAADLLNK GDPNNLLVAV QASVEKILFS
PamHNL5 201 SLDHEAGTRL TGSTFDNNGT RHAADLLNK GDPNNLLVAV QASVEKILFS

PamHNL5Gox 237 SNTSNLSAIG VIYTDSDGNS HQAFVRNGE VIVSAGTIGT PQLLLLSGVG
PamHNL5 251 SNTSNLSAIG VIYTDSDGNS HQAFVRNGE VIVSAGTIGT PQLLLLSGVG

PamHNL5Gox 287 PESYLSSLNI TVVQPNPYVG QFVYDNPRNF INILPPNPIE ASVVTVLGIR
PamHNL5 301 PESYLSSLNI TVVQPNPYVG QFVYDNPRNF INILPPNPIE ASVVTVLGIR

PamHNL5Gox 337 SDYYQVSLSS LPFSTPPFSL FPTTSYPLPN STFAHIVSQV PGPLSHGSVT
PamHNL5 351 SDYYQVSLSS LPFSTPPFSL FPTTSYPLPN STFAHIVSQV PGPLSHGSVT

PamHNL5Gox 387 LNSSSDVRIA PNIKFNYYSN STDLANCVSG MKKLGDLLRT KALEPYKARD
PamHNL5 401 LNSSSDVRIA PNIKFNYYSN STDLANCVSG MKKLGDLLRT KALEPYKARD

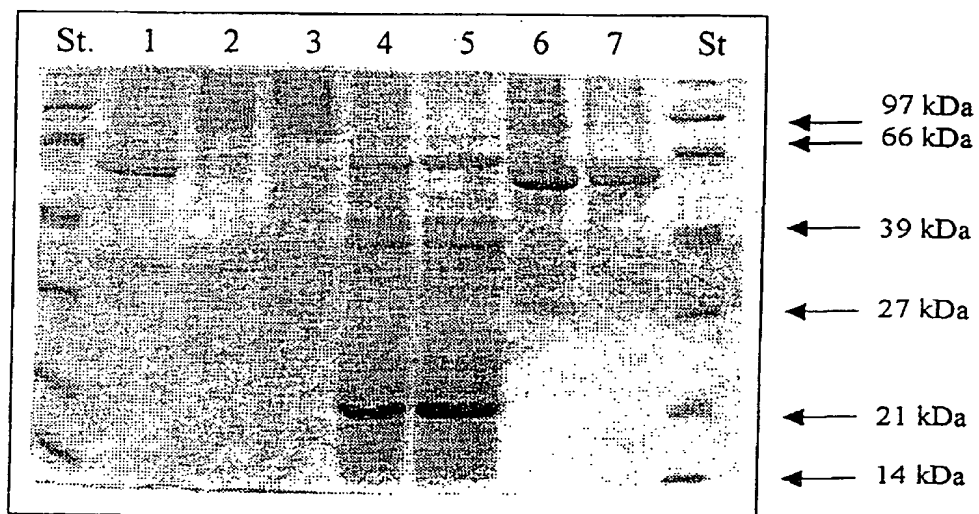
PamHNL5Gox 437 VLGIDGFNYL GVPLPENQTD DASFETFCLD NVASYWHYHG GSLVGKVLDD
PamHNL5 451 VLGIDGFNYL GVPLPENQTD DASFETFCLD NVASYWHYHG GSLVGKVLDD

PamHNL5Gox 487 SFRVMGIKAL RVVDASTFPY EPNSHPQGFY LMLGRYVGLQ ILQERS*mg*--
PamHNL5 501 SFRVMGIKAL RVVDASTFPY EPNSHPQGFY LMLGRYVGLQ ILQERS*irle*

PamHNL5Gox 535 -----
PamHNL5 551 *aihniqesm*

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Figure 7: Analysis of HNL preparations by SDS PAGE.
Details are described in example 11.



209TFO 2E29400T

Figure 8:

Nucleotide sequence of the *Prunus amygdalus* HNL1 gene obtained by PCR amplification.

ATGGAGAAATCAACAATGTCAGCTATACTGTTGGTGTATACATTTTTGTCCTCC
ATCTTCAATATTCTGAGGTCCACT
CGCTTGCCACGACTTCTGATCATGgtaaatcactcaaccgtaattcaaaacacaaaaagg-
caatcaaaaagaaaacg
gaaaaaagtgaagaaaagcagatatagacgcctgcatagatgcatgtgctatatactttaaaaactcttcgtctctt
gagatttgcagATTTTAGCTACCTGAGCTTTGCATACGACGCCACTGATCTA-
GAGTTGGAAGGATCATATGACTACGT
TATAGTTGGCGGAGGAACATCAGGGTGTCCATTGGCAGCAACTTTATCAGAAAA
ATACAAGGTGCTCGTTCTCGAAAGG
GGCAGTCTTCCGACAGCATATCCCAACGTCTTGACTGCAGATGGGTTTGTATAT
AATCTCCAGCAAGAAGATGATGGAA
AGACACCGGTGCAAAGGTTTCGTGTCCGAAGATGGTATTGATAATGTACGGGGC
AGGGTGCTCGGTGGCACAAGCATTAT
CAATGCCGGTGTCTACGCCAGAGCTAACACCTCAATCTATAGTGCATCAGGAGT
TGATTGGGACATGGATTGGTTAAT
CAGACATATGAGTGGGTTGAAGACACTATTGTGTACAAGCCAAATTCTCAATCTT
GGCAGTCTGTTACAAAACTGCAT
TCTTGGAGGCTGGTGTTTCATCCAAACCATGGATTAGTTTAGATCATGAAGAAG
GAACTAGAATTACCGGCTCAACTTT
TGACAACAAGGGAACGAGACATGCAGCTGATGAACTTCTTAATAAAGGAAACTC
TAACAACTTGCGAGTTGGAGTTCAT
GCCTCAGTAGAGAAGATCATCTTCTCCAATGCACCAGglatgttgcacatgcactcaa-
aattaataatttgcattt
taaaacactagcaggagccaaggctggaagtacgaataaaattcattatttccctggattgttgataatgatta
taagcttttctgtaatgtagGTTTGACAGCTACAGGAGTCATATATAGGGATTCTAATG-
GAACGCCTCACCAAGCATT
GTACGCAGTAAGGGAGAAGTTATCGTGAGTGCAGGGACAATTGGGACCCCTCA
ACTTCTACTACTTAGCGGTGTTGGGC

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CAGAGTCTTACCTATCATCTCTAAATATTCCAGTTGTTCTTTCCCATCCTTACGTC
GGACAGTTTCTGCATGACAATCC
TCGTAATTTTATTAACATTTTGGCCCCAAATCCAATTGAACCCACAATTGTAAGTG
YTCTAGGCATTTCAAACGATTTT
TACCAATGTTCTTTCTCGAGCTTGCCATTTACAACCTCCACCCTTCGGTTTTTTCC
CTAGTGCATCTTATCCCCTGCCAA
ATTCGACTTTTGGCTCACTTTGCTAGCAAAGTGGCAGGACCTTTATCATATGGTTC
TCTCACACTGAAATCATCCTCCAA
TGTGAGAGTCAGTCCAAATGTCAAATTTAATTACTATTCAAATCTGACAGATCTTT
CTCATTGTGTTAGCGGCATGAAG
AAGATTGGTGAACCTTTGAGCACAGACGCATTAAAACCATATAAAGTTGAAGATT
TGCCGGGTGTAGAAGGTTTTAATA
TTTTGGGAATCCCTTTGCCAAAGGACCAAACAGATGATGCAGCCTTCGAAACAT
TTTGCCGAGAATCAGTAGCCTCATA
TTGGCACTACCACGGTGGATGCCTTGTTGGAAAGGTGCTTGATGGTGATTTCCG
TGTTACAGGGATCAATGCATTACGC
GTTGTTGATGGCTCAACATTCCCTTACACACCAGCGAGCCACCCTCAGGGCTTC
TATCTGATGTTAGGGAGGtatgta
caaattctcaataatttgggtgagtgacctgttgtaatagaactctatgccatatttctttctcatcctttcca
ttttgtgccatgggcagGTATGTGGGCATTAAAATTCTGCAAGAAAGATCAGCTTCA-
GATCTAAAAATCTTGGATTCC
CTCAAGTCAGCAGCATCCTTGTTCTTTAACT

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Figure 9:

Amino acid sequence of *Prunus amygdalus* hydroxynitrile lyase (HNL1), derived from the nucleotide sequence of the HNL1 gene.

MEKSTMSAILLVLYIFVLHLQYSEVHSLATTSDHDFSYSLSFAYDATDLELEGSY
DYVIVGGGTSGCPLAATLSEKYKVLVLERGSLPTAYPNVLTADGFVYNLQQE
DDGKTPVERFVSEDGIDNVRGRVLGGTSIINAGVYARANTSIYSASGVDWDM
DLVNQTYEWVEDTIVYKPNSQSWQSVTKTAFLEAGVHPNHGFSLDHEEGTRI
TGSTFDNKGTRHAADELLNKGNSNNLRVGVHASVEKIIFSNAPGLTATGVIYR
DSNGTPHQAFVRSKGEVIVSAGTIGTPQLLLLSGVGPESYLSSLNIPVVLSPY
VGQFLHDNPRNFINILPPNPIEPTIVTVLGISNDFYQCSFSSLPFTTPPFGLFFPS
ASYPLPNSTFAHFASKVAGPLSYGSLTLKSSSNVRVSPNVKFNYYSNLTDLSHC
VSGMKKIGELLSTDALKPYKVEDLPGVEGFNILGIPLPKDQTDAAAFETFCR
ESVASYWHYHGGCLVGKVLGDGDFRVTGINALRVVDGSTFPYTPASHPQGFYL
MLGRYVGIKILQERSASDLKILDSLKSAASLV

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